REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	19 April 1999	Final Report 1 June 1993 - 30 September t999

4. TITLE AND SUBTITLE

Digestive Kinetics Determines Bioavailability of Pollutants

5. FUNDING NUMBERS

N00014-93-1-1077

6. AUTHOR(S)

Peter A. Jumars (and Lawrence M. Mayer)

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)

School of Oceanography
Campus Box 357940
University of Washington
Seattle, WA 98195-7940

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAMES(S) AND ADDRESS(ES)

Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

a. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution Unlimited

12. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 words)***

We assayed digestive capabilities of marine deposit feeders (animals that eat sediments) by using fluorescently tagged substrates and contact-angle measurements of surfactancy. Polychaetes on average showed higher enzyme activities and surfactancy than echinoderms. We found that surfactants produced by deposit feeders substantially enhance their abilities to solubilize hydrophobic pollutants such as polycyclic aromatic hydrocarbons (PAHs). Amounts solubilized were consistent with incorporation into micelles of the surfactant. Kinetics of PAH uptake could be explained by passive diffusion. We also found that the digestive strategies of deposit feeders often produce concentrations of proteins (digestive enzymes plus products of protein digestion) that are sufficient to solubilize metals. Histidine residues in these proteins were found to be critical for copper binding.

14. SUBJECT TERMS

Deposit feeding, PAH, Metal, Sediments, Polychaetes, Echinoderms

15. NUMBER OF PAGES
4 (four)

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited (UL)

FINAL REPORT

Grant #: N00014-93-1-1077

PRINCIPAL INVESTIGATOR: Dr. Peter A. Jumars

INSTITUTION: University of Washington

PROJECT TITLE: Digestive Kinetics Determines Bioavailability of Pollutants

REPORTING PERIOD: 1 July 1993 - 30 September 1998

AWARD PERIOD: 1 July 1993 - 30 September 1998

OBJECTIVES: (1) Determine digestive physiologies of deposit feeders; (2) determine the fraction of total pollutants in sediments released during incubation of polluted sediments with digestive fluid of deposit feeders; (3) examine partitioning of pollutants in sediments, mechanism of digestive fluid solubilization, and design an in vitro method to measure their bioavailability.

APPROACH: (1) We used enzyme and surfactant assays to study digestive capabilities of a variety of benthic invertebrate animals for lipid component of sediments; identify surfactant compounds in animal guts and digestive products of lipid digestion; (2) extracted gut juices from benthic invertebrates and incubated them with polluted sediments, followed by measurement of pollutants released during the incubation; (3) after determining patterns of digestive agents in various animals, designed a cocktail of commercially available enzymes and surfactants that can be used to mimic the pollutant release kinetics found in part (2), and explored phase associations of sedimentary pollutants. (4) We sought closure by comparing observed results with predictions from chemical reactor-theory models of animal digestion.

ACCOMPLISHMENTS: We surveyed the digestive enzyme and surfactant activities of 18 benthic invertebrate animals. We focused on deposit feeders from the U.S. east and west coasts, but included carnivores, herbivores, and suspension feeders for context. Enzyme and surfactant activities varied across both functional and phyletic categories. Polychaetes had higher enzyme activities than echinoderms, and detritivorous polychaetes had higher protease:lipase ratios than herbivorous and carnivorous species. Intense surfactant activities were found in deposit feeders. Less intense surfactancy was found in carnivores, herbivores and suspension feeders. Surfactants from Arenicola marina were consisted of a C9 fatty acid connected via amide bond to an amino acid. Fatty acids show branching and saturation. Feeding experiments with an omnivorous polychaete showed that varying diet could induce changes in protease:lipase ratio similar to cross-phyletic patterns. Presence or absence of sediment in the diet had no effect on the surface

tension of digestive fluid, but sediment did induce higher surfactant concentrations, as indicated by micelle formation. Thus diet can affect digestive chemistry that can in turn affect contaminant solubilization. Gut fluids had high dissolved organic matter concentrations, with some polychaetes showing dissolved amino acids alone of 5-10%. Amino acids made up roughly one-half of the dissolved organic matter. Lipids were much less concentrated. Circumstantial evidence and experimental work showed that animals can concentrate dissolved materials (e.g., nutritional amino acids, PAH, and radiolabelled polymers) in gut fluids by retaining fluids relative to solids. Such enrichments will affect subsequent absorption.

Dissolved trace-metal concentrations in digestive fluids were extremely high, with several species having ppm levels. Levels of some metals were proportional to amino acid concentrations. Metal enrichments in gut fluids, relative to sediments, showed the Irving-Williams order, consistent with soft ligands (e.g., amino acids) as the solubilizing agents.

Metals in contaminated sediments were released during incubation with digestive fluid to a much greater extent than in incubation with seawater. Kinetics of release to digestive fluid were complex among metals and sediments, including even biphasic patterns with opposite signs (adsorption followed by release). For highly contaminated sediments, the release rate for a metal like Cu is slow enough that incomplete solubilization will occur during gut passage of sediment through the animal. These kinetic patterns will affect the animals' ability to bioaccumulate metals.

The amount of Cu solubilized by different molecular-weight fractions of digestive fluid is related to their peptide content. Solubilization is due to complexation by proteins rather than their enzymatic activity. We mimicked Cu release with solutions of off-the-shelf proteins at concentrations found in digestive fluid. Histidine residues were critical for Cu binding by digestive fluids. Both high molecular-weight proteins, representing enzymes secreted by the animal, and lower molecular-weight peptides, representing nutritional material solubilized from sediment, were important in releasing Cu from contaminated sediments. Low molecular-weight fractions were found to be more effective solubilizing agents, per amino acid residue, likely due to greater exposure to solution. Ion-specific electrodes were used to determine conditional binding constants between Cu and gut ligands, and ranged from 10^{-4} to 10^{-14}, consistent with known histidine affinities for Cu.

Comparison of digestive fluid extraction with the acid-volatile sulfide (AVS) method showed agreement. Digestive solubilization of borderline metals (Cu, Cd, Ni, Zn, Pb) occurred only when these metals were present in concentrations in excess of the AVS, consistent with the relative binding affinities of histidine vs. sulfide.

Solubilization of Cu from sediment can result in inactivation of digestive fluid enzymes. Inactivation occurs at similar ratios of Cu to gut amino acids, across enzyme type and animal species. Polycyclic aromatic hydrocarbons (PAH) were also found to be solubilized by digestive fluids much more than by seawater. PAH solubilization was found to depend strongly on the solid-solution ratio and sedimentary organic carbon content. At in vivo solid-solution ratios, PAH solubilized by digestive fluid was inversely dependent on sedimentary OC concentration. Increasing the solid-solution ratio decreased the proportion of total PAH extractable by digestive fluid. These two sets of results imply a strong, fugacity-driven partitioning between digestive fluid and sediment. Using dilution experiments that tested for PAH solubility above and below the critical micelle concentration (CMC) of the surfactants in gut fluids, we established an important role of surfactants in PAH solubilization. Solubilities of heavy and light PAH were similar in gut fluid micelles, presumably due to similar limitation by micellar volume. Proteins may also solubilize PAH and other hydrophobic materials (e.g, methyl Hg). PAH solubilization from contaminated sediments is much less than from pure PAH solids, implicating competition for micellar space from other sedimentary lipids. PAH solubilization from highly contaminated sediment is also limited by saturation of the micelles. Hence bioavailability from such sediments will be more a function of surfactant secretion by the animal than of inherent solubility from the sediment matrix. Repeatedly incubating sediment with fresh batches of gut fluid released similar amounts of PAH, consistent with saturation behavior. The kinetics of PAH solubilization were found to be rapid, reaching completion within a gut residence time. Absorption of PAH by gut walls is dominantly by passive diffusion.

Very recently, we have discovered an apparent association of countercurrent flow with the concentration of digestive products and surfactants near the midgut-foregut junction of some deposit feeders. Analysis of mixing patterns in the gut also challenges the idea of an "unstirred layer" adjacent to the gut wall, and both these developments have necessitated revisions of the reactor-theory description of digestion in deposit feeders (Rangel and Jumars, in preparation).

CONCLUSIONS: Digestive fluid contains agents – e.g., amino acids and surfactants – that significantly increase exposure of benthic animals to contaminants during digestion. Variations in bio/chemical properties among animal species and among sediments can explain patterns of bioavailability among animal-sediment combinations. The interactive chemistry of these two "reactants" governs bioavailability.

SIGNIFICANCE: Lack of understanding of bioavailability is the major hindrance in the application of science to contaminant management issues. This study provides a scientific basis for the failure of current regulatory models to explain animal exposure to sedimentary

contaminants in harbors. It provides a basis for more accurate understanding and routine determination of contaminant bioavailability and hence risk assessment.

AWARD INFORMATION: G. Evelyn Hutchinson Medal of the American Society of Limnology and Oceanography 1994; Fellow of the American Geophysical Union 1995

PUBLICATIONS:

Mayer, L., Z. Chen, R. Findlay, J. Fang, S. Sampson, L. Self, P. Jumars, C. Quetél, & O. Donard, 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion, Envir. Sci. Tech., 30:2641-2645.

Mayer, L.M., L.L. Schick, R.F.L. Self, P.A. Jumars, R. H. Findlay, Z. Chen, S. Sampson, 1997. Digestive environments of benthic macroinvertebrate guts: Enzymes, surfactants, and dissolved organic matter, J. Mar. Res., 55:1-30.

Chen, Z. and L. Mayer, 1998. Mechanisms of Cu solubilization during deposit-feeding Envir. Sci. Tech., 32:770-775.

Chen, Z. and L. Mayer, 1998, Digestive proteases of the lugworm, Arenicola marina, inhibited by Cu from contaminated sediments, Envir. Toxic. Chem., 17:433-438.

Weston, D. and L. Mayer, 1998. In vitro digestive fluid extraction as a measure of the bioavailability of sediment-associated polycyclic aromatic hydrocarbons: Sources of variation and implications for partitioning models, Envir. Toxic. Chem., 17:820-829.

Weston, D. and L. Mayer, 1998. Comparison of in vitro digestive fluid extraction and traditional in vivo approaches as measures of polycyclic aromatic hydrocarbon bioavailability from sediments, Envir. Toxic. Chem., 17:830-840.

Chen, Z. and L. Mayer, 1999. Assessment of sedimentary Cu availability: A comparison of biomimetic and AVS approaches, Envir. Sci. Tech., 33:650-652.

Chen, Z., and L. Mayer 1999. Sedimentary metal bioavailability determined by the digestive constraints of marine deposit feeders: gut retention time and dissolved amino acids, Mar. Ecol. - Prog. Ser., 176:139-151.

Bock, M. & L. Mayer, in press. Digestive plasticity of the marine benthic omnivore Nereis virens, J. Exp. Mar. Biol. Ecol.

Lawrence, A.L., K.M. McAloon, R.P. Mason, and L.M. Mayer, in press. Intestinal solubilization of particle-associated organic and inorganic mercury as a measure of bioavailability to benthic invertebrates, Envir. Sci. Tech.

Jumars, P.A., and C. Martínez del Rio. The tau of continuous feeding on simple foods. *Physiol. Zool.*, in review.

Jumars, P.A. Animal guts as ideal chemical reactors: maximizing absorption rates. *Am. Nat.*, in review.

Jumars, P.A. Animal guts as non-ideal chemical reactors: partial mixing and axial variation in absorption kinetics. in preparation for submission to *Am. Nat.*, in review.